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PATENT & TRADEMARK OFFICE

Docket No.: AVZ-020CNRCE
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Rima Kaddurah-Daouk

Application No.: 09/852,966 Confirmation No.: 5588

Filed: May 10, 2001 Art Unit: 1618

For: USE OF CREATINE OR CREATINE COMPOUNDS FOR SKIN PRESERVATION Examiner: V. Y. Kim

APPEAL BRIEF

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

As indicated in the Notice of Appeal filed on April 7, 2006, Appellant hereby appeals the final decision of the Examiner in the above-identified application rejecting the subject matter of the pending claims. For the reasons set forth in this brief, Appellant respectfully requests the Board of Patent Appeals and Interferences to reverse the Examiner's final rejection of the claimed subject matter.

As required under § 41.37(a), this brief is filed more than two months after the Notice of Appeal filed in this case, and is in furtherance of said Notice of Appeal.

The fees required under § 41.20(b)(2) are dealt with in the accompanying Transmittal of Appeal Brief.

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This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1206:

I. REAL PARTY IN INTEREST

The real party in interest for this appeal is Avicena Group, Inc.

II. RELATED APPEALS, INTERFERENCES, AND JUDICIAL PROCEEDINGS

There are no other appeals, interferences, or judicial proceedings known to Appellant, Appellant's legal representative or the assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

A. Total Number of Claims in Application

There are 17 claims pending in application.

B. Current Status of Claims

1. Claims canceled: 1-67, 71, 74, 86 and 87
2. Claims withdrawn from consideration but not canceled: none
3. Claims pending: 68-70, 72, 73, 75-85 and 88
4. Claims allowed: none
5. Claims rejected: 68-70, 72, 73, 75-85, and 88

C. Claim on Appeal

The claims on appeal are claims 68-70, 72, 73, 75-85, and 88.

IV. STATUS OF AMENDMENTS

An Amendment and Response to Office Action was filed on June 27, 2003 in response to the Office Action dated June 4, 2003 and was entered.

An Amendment and Response to Final Office Action Pursuant to 37 C.F.R. §1.116 and a Notice of Appeal was filed on May 17, 2004 in response to the final office action dated November 26, 2003. The response was not entered.

A Request for Continued Examination Pursuant to 37 C.F.R. §1.114 was filed on August 16, 2004.

An Amendment and Response to Office Action was filed on July 11, 2005 in response to the office action dated January 1, 2005 and was entered.

Appellant did not file an Amendment and Response Pursuant to 37 C.F.R §1.116 in response to the final office action dated October 7, 2005. A Notice of Appeal was filed on April 7, 2006.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Appellant's invention pertains, at least in part, to methods for protecting skin tissue against age related damage or insults, such as harmful UV radiation, stress and fatigue, by preserving energy pools and protecting against free radicals and oxidative stress (see, page 2, lines 19-28).

One aspect of the invention provides a method of increasing energy reserves in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue, by administering to the subject an effective amount of creatine or a salt thereof (see, *e.g.*, page 4, lines 18-20).

Another aspect of the invention provides a method for sustaining energy production in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue, by administering to the subject an

effective amount of creatine or a salt of thereof (see, *e.g.*, page 20, lines 9-12, and page 22, lines 6-8).

A further aspect of the invention provides a method for modulating energy flow in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue, by administering to the subject an effective amount of creatine or a salt thereof (see, *e.g.*, page 20, lines 19-22).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellant presents the following issues for review:

1. Whether claims 68-70, 75-80, 84 and 85 are properly rejected under 35 U.S.C. §103(a) as obvious over Yu *et al.* (U.S. Patent No. 5,702,688; Appendix B) in view of Kaddurah-Daouk *et al.* (U.S. Patent No. 5,324,731; Appendix C) and Kaddurah-Daouk *et al.* (International Application Publication No. WO 96/14063; Appendix D).

2. Whether claims 68-70, 75-85 and 88 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Le Fur *et al.* (U.S. Patent No. 5,256,649; Appendix E) in view of Carniglia (U.S. Patent No. 4,871,718; Appendix F) and Kaddurah-Daouk *et al.*(International Application Publication No. WO 96/14063; Appendix D) or Kaddurah-Daouk *et al.* (U.S. Patent No. 5,321,030; Appendix G).

VII. GROUPING OF CLAIMS

Claims 68-70, 72, 73, 75-85, and 88 are Appellant's principal claims on appeal. Claim 68 is an independent claim drawn to a method of increasing energy reserves in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue by administering to the subject an effective amount of creatine or a salt thereof, such that the energy reserves in the skin of the subject is increased.

Claim 69 is an independent claim drawn to a method for sustaining energy production in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue by administering to the subject an effective amount of creatine or a salt thereof, such that the energy production in the skin of the subject is sustained.

Claim 70 is an independent claim drawn to a method for modulating energy flow in the skin of a subject suffering from a skin disorder associated with free radicals, aging, sun radiation, stress or fatigue by administering to the subject an effective amount of creatine or a salt thereof, such that the energy flow in the skin of the subject is modulated.

Claims 72, 73, 75-85 and 88 depend from each of the above-described independent claims.

Claim 72 is directed to creatine monohydrate.

Claim 73 is directed to creatine citrate.

Claim 75 is directed to the co-administration to the subject an effective amount of a skin preserving agent. Claim 76, which depends from claim 75, is directed to an antioxidant. Claim 77, which depends from claim 76, is directed to CoQ_{10} or vitamin E. Claim 78, which also depends from claim 76, is directed to an energy-enhancing agent. Claim 79, which depends from claim 78, is directed to ATP, nicotinamide and pyruvate. Claim 80, which depends from claim 75, is drawn to a vitamin or vitamin precursor. Claim 81, which depends from claim 80, is drawn to a vitamin selected from the group consisting of E, C, B5, B6 and B9.

Claim 82 is directed to the co-administration to the subject a sunscreen or sunblock. Claim 83, which depends on claim 82, is drawn to zinc oxide or titanium dioxide.

Claim 84 is drawn to the co-administration of a pharmaceutical carrier suitable for topical administration.

Claim 85 is directed to a human.

Claim 88 is directed to skin wrinkles.

The rejected claims do not stand or fall together for the reasons set forth below.

VIII. ARGUMENTS

A. Rejection of Claims 68-70, 75-80, 84 and 85 Under 35 U.S.C. §103(a)

The Examiner rejects claims 68-70, 75-80, 84 and 85 as obvious over Yu *et al.* (U.S. Patent No. 5,702,688, hereinafter "the '688 patent;" Appendix B) in view of Kaddurah-Daouk *et al.* (U.S. Patent No. 5,324,731, hereinafter "the '731 patent;" Appendix C) and Kaddurah-Daouk *et al.* (International Application Publication No. WO 96/14063, hereinafter "the '063 application;" Appendix D).

The present claims are directed to methods for increasing energy reserves in the (claim 68), sustaining energy production in the skin of a subject (claim 69) or modulating energy flow in the skin of a subject (claim 70) in the skin of a subject suffering from a skin disorder by administering to a subject creatine or a salt thereof, such that energy reserves are increased, the energy production is sustained or the energy flow in modulated in the skin. Claims 75, which depends on any one of claims 68-70 is directed to the co-administration an effective amount of a skin preserving agent. Claim 76, which depends from claim 75, is drawn to a skin preserving agent that is an antioxidant. Claim 77, which depends from claim 76 is directed to the antioxidants vitamin E and CoQ_{10}. Claim 78, which depends from claim 76, is directed to a skin preserving agent that is an energy-enhancing agent. Claim 79, which depends from claim 78, is drawn to the energy-enhancing agents ATP, nicotinamide and pyruvate. Claim 80, which depends on claim 75, is directed to a skin preserving agent that is a vitamin or a vitamin precursor. Claim 84, which depends on any one of claims 68-70, is directed to the co-administration of a pharmaceutical carrier suitable for topical administration. Claim 85, which depends from any one of claims 68-70, is directed to a subject that is a human.

The Examiner asserts that when the '688 patent, the '731 patent and the '063 patent "are combined together, the underlying mechanism (*i.e.*, modulating skin cell energy using creatine compounds) is clearly present in the treating of skin aging and wrinkle[s] by administering a creatine compound." The Examiner further asserts that

> [i]t is noted that creatine is also found in skin cells as well as brain, heart and muscle cells that is conventionally known knowledge at the time the invention was made...It is readily apparent to any skilled artisan that the energy level modulation by creatine supplement is not limited to the brain,

> muscle or heart cells but any cells that are associated with creatine kinase/creatine phosphate energy system. Thus, one would have been motivated to use a creatine compound to modify intercellular energy...in the skin cell to treat the diseases associated with imbalanced creatine kinase level.

Specifically, the Examiner is of the opinion that the primary reference, the '688 patent, teaches a treatment of abnormal skin conditions using an amphoteric compound. The Examiner acknowledges that Appellant's claims differ from the '688 patent "in that the claims require increasing energy reserve, sustaining energy production and modulating energy flow in the skin."

The '688 patent teaches compositions for and methods of treating skin disorders using an amphoteric composition comprising an amphoteric compound and at least one of an alpha hydroxyacid or an alpha ketoacid. While the '688 patent discloses that creatine is an example of an amphoteric compound, the '688 patent emphasizes that the active compounds are the alpha hydroxyacids and the alpha ketoacids, not the amphoteric compounds. The amphoteric compounds are merely included in the amphoteric composition to modulate the pH of the composition and to control the release of the "active ingredients" (*e.g.*, alpha ketoacids and alpha hydroxy acids). Therefore, the '688 patent ***fails to teach or suggest that an amphoteric compound (e.g., creatine) would be useful in any other capacity other than to balance the pH and enhance the ability of the alpha ketoacids and the alpha hydroxyacids to penetrate the skin.***

The Examiner takes the position that the '731 patent teaches "a creatine (or its salts) and its use in the treatment of metastasis of epithelial cells via modifying energy level" and that the '731 patent further "teaches energy balance using creatine kinase in the treatment of other diseases such as psoriasis, wound healing, neurological disorders and cerebrovascular diseases."

The '731 patent also fails to provide any teaching or suggestion which would have led one of ordinary skill in the art to the claimed invention. Specifically, the '731 patent describes methods of inhibiting growth, transformation and/or metastasis of mammalian cells, where the activity of at least one purine metabolic enzyme (*e.g.*, creatine kinase) is elevated, by administering a drug that directly or indirectly reduces the velocity of the enzymatic activity. The '731 patent further describes that reducing the

activity of creatine biosynthetic enzymes affects the velocity of the reaction of creatine kinase ***by limiting substrates of creatine kinase (e.g., creatine)*** (see, column 19, lines 37-42). Moreover, the '731 patent teaches the design and use of drugs that are structural analogs of creatine, where the ***analogs differ from creatine*** by "substitution, chain extension and/or cyclization," such that the analogs exhibit "greater specificity for the enzyme, enhanced stability, enhanced uptake into cells, tighter binding to the enzyme or ***better inhibitory activity*** (see, column 20, lines 45-52 and column 21, lines 40-42)." And although the '731 patent suggests that the disruption of cellular energy balance may be important in diseases or disorders, such as psoriasis, arthritis and wound healing, where levels of creatine kinase B are elevated, the '731 patent teaches ***away*** from using creatine or a salt thereof for the treatment of these disorders because ***creatine is a substrate for creatine kinase.*** Thus, the administration of creatine or a salt thereof would be expected to increase the velocity of the reaction of creatine kinase.

In contrast, Appellant's claim methods for increasing energy reserves, sustaining energy production and modulating energy flow in the skin by administering to a subject an effective amount of ***creatine or a salt thereof.*** Accordingly, based on the teaching of the '731 patent, the subject matter presently claimed by Appellant would not have been obvious to one of ordinary skill in the art. Indeed, the '731 patent fails to provide any motivation at all to administer creatine or a salt thereof to a subject suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue.

With regard to the '063 application, the Examiner asserts that the '063 application

> teaches a treatment of disesase (*e.g.*, neurological diseases) which are caused by abnormalities in an energy state, wherein the induction or inhibition of creatine kinase is a cause or a consequence of disease and modulating its activity would modulate energy flow and affect cell function

and that the '063 application

> specifically teaches that creatine (or its salts) is used for modifying energy of cells in stress via increasing energy reserve, sustaining energy production and modulating energy flow.

The '063 application, alone or in combination with the '731 patent, fails to overcome the deficiencies of the primary reference. The '063 application teaches

methods of treating diseases of the nervous system (*e.g.*, Alzheimer's disease, Parkinson's disease, Huntington's disease and the like) in a subject by administering to the subject an amount of one or more compounds (*e.g.*, creatine, phosphocreatine or analogs thereof) which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system (*e.g.*, creatine, creatine phosphate, creatine kinase and transporter of creatine) sufficient to prevent, reduce or ameliorate the symptoms of the disease. Further, the '063 application discloses ***cyclocreatine***, not creatine or a salt thereof, "modifies the flow of energy of cells in stress (see, page 40, lines 6-8)." Moreover, although the '063 application discloses that the function of the ***creatine kinase/phosphocreatine system*** includes the regeneration of energy in cells and that modulation the activity of creatine kinase would modulate energy flow and affect cell function, there is no teaching or suggestion of methods for increasing energy reserves, sustaining energy production and ***modulating energy flow in the skin*** by administering to a subject an ***effective amount of creatine or a salt thereof*** to a subject suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue. Accordingly, based on the teaching of the '063 application, alone or in combination with the '688 patent or the '731 patent, the subject matter presently claimed by Appellant would not have been obvious to one of ordinary skill in the art.

In sum, none of the cited references, either alone or in combination, provide any teaching or suggestion which would have motivated one of ordinary skill in the art to use the methods of increasing energy reserves, sustaining energy production and modulating energy flow in a subject suffering from a skin disorder, as claimed by Appellant. Therefore, Appellant respectfully submits that claims 68-70, 75-80, 84 and 85 are patentable over the '688 patent in view of the '731 patent and the '063 application.

B. Rejection of Claims 68-70, 75-85 and 88 Under 35 U.S.C. §103(a)

The Examiner rejects claims 68-70, 75-85 and 88 under 35 U.S.C. §103(a) as being unpatentable over Le Fur *et al.* (U.S. Patent No. 5,256,649; hereinafter "the '649 patent;" Appendix E) in view of Carniglia (U.S. Patent No. 4,871,718; hereinafter "the '718 patent;" Appendix F) and the '063 application or Kaddurah-Daouk *et al.* (U.S. Patent No. 5,321,030; hereinafter "the '030 patent;" Appendix G).

Claims 68-70, 75-80, 84 and 85 are described immediately above in section A. Claim 81, which depends on claim 80, is directed to a vitamin selected from the group consisting of E, C, B5, B6, and B9. Claim 82, which depends on any one of claims 68-70 is directed to the co-administration of a sunscreen or a sunblock. Claim 83, which depends on claim 82, is directed to a sunscreen or sunblock with is zinc oxide or titanium dioxide. Claim 88, which depends on any one of claims 68-70, is directed to a skin disorder that is skin wrinkles.

The Examiner takes the position that "it would have been obvious to one of ordinary skill in the art to substitute [an] ATP generating systems with creatine (or its salts) when Le Fur [the '649 patent] is taken in view of Carniglia [the '718 patent] and Kaddurah-Daouk *et al.* [the '030 patent or the '063 application] because both Carniglia and Kaddurah-Daouk *et al.*'s patent together remedy the deficiencies of Le Fur's."

The Examiner further asserts that

> the modification of cellular energy level via increasing energy reserve, sustaining energy production and modulating energy flow is [an] inherently possessed feature where the intracellular energy metabolism in [the] skin cell is modified by creatine supplement because creatine is also found in [the] skin cell as well as brain, heart and muscle cells...

The '649 patent is directed to a cosmetic composition for combating aging of the skin, by administering an ademetionine (SAMe) generating system. Specifically, the composition disclosed in the '649 patent requires betaine, ATP or an ATP generating system, a magnesium salt, and a potassium salt and that ***the combination of the betaine and the ATP generating system*** are important to generate ademetionine *in situ* to treat the skin. Moreover, the methods taught by the '649 patent are limited to methods of

treating the skin using ***ademetionine*** or precursors thereof. The '649 patent does not teach or suggest any methods using an ***ATP generating system alone***, let alone ***methods of administering creatine*** to the skin of a subject who is suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue, as claimed by Applicant.

The '718 patent fails to overcome the deficiencies of the primary reference. Specifically, the '718 patent teaches compositions comprising amino acids (*e.g.*, metabolic precursors of ATP), metabolites (*e.g.*, inositol), electrolytes (*e.g.*, magnesium phosphate) and a pentose sugar (*e.g.*, d-ribose), which are useful for increasing ATP levels, physical performance levels and the rate of wound repair. Specifically, the '718 patent discloses that the rate of localized wound contraction produced by myofibroblasts is dependent on the amount of ATP available as an intracellular source. While the '718 patent discloses that ATP serves as an energy source for other wound repair processes, such as granulation of the wound by fibroblasts, gluconeogenesis, protein synthesis and epithelialization, the '718 patent fails to teach or suggest ***methods for increasing energy reserves, sustaining energy production or modulating energy flow in the skin by administering to a subject an effective amount of creatine or a salt thereof*** to a subject suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue. Accordingly, based on the teaching of the '718 patent, alone or in view of the primary reference, the subject matter presently claimed by Appellant would not have been obvious to one of ordinary skill in the art.

The '063 application, alone or in combination with the '718 patent, fails to overcome the deficiencies of the primary reference. The '063 application, as described immediately above in section A, teaches methods of treating diseases of the nervous system (*e.g.*, Alzheimer's disease, Parkinson's disease, Huntington's disease and the like) in a subject by administering to the subject an amount of one or more compounds (*e.g.*, creatine, creatine phosphate and analogs thereof) which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate the symptoms of the disease. As described above, although the '063 application discloses that the function of the ***creatine kinase/phosphocreatine system*** includes the regeneration of energy in cells and that

modulation the activity of creatine kinase would modulate energy flow and affect cell function, there is no teaching or suggestion of methods for increasing energy reserves, sustaining energy production and ***modulating energy flow in the skin*** by administering to a subject an ***effective amount of creatine or a salt thereof*** to a subject suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue. Accordingly, based on the teaching of the '063 application, alone or in combination with the '649 patent or the '718 patent, the subject matter presently claimed by Appellant would not have been obvious to one of ordinary skill in the art.

The '030 patent, alone or in combination with the '718 patent, also fails to provide any teaching or suggestion which would have led one of ordinary skill in the art to the claimed invention. The '030 patent teaches the use of ***creatine analogs*** (*e.g.*, cyclocreatine) as anti-viral agents. Specifically, the '030 patent teaches that creatine analogs can be used for treatment of a variety of infections caused by DNA viruses (*e.g.*, adenoviruses, herpes simplex virus, cytomegalovirus, etc...) and RNA viruses (*e.g.*, influenza). The '030 further teaches that because creatine kinase plays an important role in controlling the flow of energy inside a cell, the induction of creatine kinase by the virus might facilitate the generation and release of cellular energy reserves required for stages of viron replication and production. Accordingly, the inhibition of creatine kinase or interference with the normal activity of creatine kinase may block the production of a progeny virus. Therefore, similar to the '731 patent described in section A immediately above, the '030 patent teaches ***away*** from the use of creatine or a salt thereof for the treatment of viruses because ***creatine is a substrate for creatine kinase.*** Thus, the administration of creatine or a salt thereof would be expected to increase the rate of the reaction of creatine kinase, thus increasing the energy reserves for use by the virus. Accordingly, the '030 patent, alone or in combination with the '718 patent or the '649 patent, would not have provided motivation for the skilled artisan to conceive of the present invention.

In sum, none of the cited references, either alone or in combination, provide any teaching or suggestion which would have motivated one of ordinary skill in the art to use the methods of increasing energy reserves, sustaining energy production and modulating energy flow in a subject suffering from a skin disorder, as claimed by Appellant.

Therefore, Appellant respectfully submits that claims 68-70, 75-85 and 88 are patentable over the '649 patent in view of the '718 patent and the '063 application or the '030 patent.

IX. CLAIMS

A copy of the claims involved in the present appeal is attached hereto as Appendix A.

X. EVIDENCE

No evidence pursuant to §§ 1.130, 1.131, or 1.132 is being submitted. However, evidence entered by or relied upon by the examiner is being submitted, as indicated immediately below and in the attached Appendices B-G.

Appendix B is a copy of U.S. Patent No. 5,702,688 (the '688 patent) to Yu *et al.*, originally cited by Appellant in an Information Disclosure Statement received by the U.S. Patent and Trademark Office on October 20, 2004.

Appendix C is a copy of U.S. Patent No. 5,324,731 (the '731 patent) to Kaddurah-Daouk *et al.* originally cited by Appellant in an Information Disclosure Statement received by the U.S. Patent and Trademark Office on April 21, 2003.

Appendix D is a copy of International Application Publication No. WO 96/14063 to Kaddurah-Daouk *et al.* originally cited by Appellant in an Information Disclosure Statement received by the U.S. Patent and Trademark Office on April 21, 2003.

Appendix E is a copy of U.S. Patent No. 5,256,649 (the '649 patent) to Le Fur *et al.* originally cited in the Office Action mailed from the U.S. Patent and Trademark Office on November 26, 2005.

Appendix F is a copy of U.S. Patent No. 4,871,718 (the '718 patent) to Carniglia originally cited in the Office Action mailed from the U.S. Patent and Trademark Office on January 1, 2005.

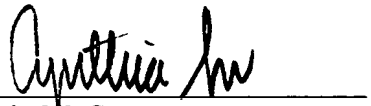
Appendix G is a copy of U.S. Patent No. 5,321,030 (the '030 patent) to Kaddurah-Daouk *et al.* originally cited by Appellant in an Information Disclosure Statement received by the U.S. Patent and Trademark Office on April 21, 2003.

XI. RELATED PROCEEDINGS

No related proceedings are referenced in II. above, or copies of decisions in related proceedings are not provided, hence no Appendix regarding related proceedings is included.

Dated: November 7, 2006

Respectfully submitted,

By 

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APPENDIX A

Claims Involved in the Appeal of Application Serial No. 09/852,966

Claims 1- 67 **(Cancelled)**

68. **(Previously Presented)** A method for increasing energy reserves in the skin of a subject, comprising administering to said subject an effective amount of creatine or a salt thereof, such that the energy reserves in the skin of said subject is increased, wherein said subject is suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue.

69. **(Previously Presented)** A method for sustaining energy production in the skin of a subject, comprising administering to said subject an effective amount of creatine or a salt thereof, such that energy production the skin of said subject is sustained, wherein said subject is suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue.

70. **(Previously Presented)** A method for modulating energy flow in the skin of a subject, comprising administering to said subject an effective amount of creatine or a salt thereof, such that the energy flow in the skin of said subject is modulated, wherein said subject is suffering from a skin disorder associated with free-radicals, aging, sun radiation, stress or fatigue.

71. **(Cancelled)**

72. **(Previously Presented)** The method of claim 68, 69, or 70, wherein said creatine salt is creatine monohydrate.

73. **(Previously Presented)** The method of claim 68, 69, or 70, wherein said creatine salt is creatine citrate.

74. **(Cancelled)**

75. **(Previously Presented)** The method of any one of claims 68-70, further comprising co-administering to said subject an effective amount of a skin preserving agent.

76. **(Previously Presented)** The method of claim 75, wherein said skin preserving agent is an antioxidant.

77. **(Previously Presented)** The method of claim 76, wherein said antioxidant is CoQ_{10} or vitamin E.

78. **(Previously Presented)** The method of claim 76, wherein the skin preserving agent is an energy-enhancing agent.

79. **(Previously Presented)** The method of claim 78, wherein said energy enhancing agent is selected from the group consisting of ATP, nicotinamide and pyruvate.

80. **(Previously Presented)** The method of claim 75, wherein said skin preserving agent is a vitamin or a vitamin precursor.

81. **(Previously Presented)** The method of claim 80, wherein said vitamin is selected from the group consisting of E, C, B5, B6, and B9.

82. **(Previously Presented)** The method of any one of claims 68-70, further comprising the coadministration of a sunscreen or sunblock.

83. **(Previously Presented)** The method of claim 82, wherein said sunscreen or sunblock is zinc oxide or titanium dioxide.

84. **(Previously Presented)** The method of any one of claims 68-70, further comprising the coadministration of a pharmaceutical carrier suitable for topical administration.

85. **(Previously Presented)** The method of any one of claims 68-70, wherein said subject is a human.

86. **(Cancelled)**

87. **(Cancelled)**

88. **(Previously Presented)** The method of claim 68, 69, or 70, wherein said skin disorder is skin wrinkles.

Appendix D

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/195, 31/66, 31/675

A1

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Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: USE OF CREATINE OR CREATINE ANALOGS FOR THE TREATMENT OF DISEASES OF THE NERVOUS SYSTEM

(57) Abstract

The present invention relates to the use of creatine compounds including creatine, creatine phosphate or analogs of creatine, such as cyclocreatine, for treating diseases of the nervous system. Creatine compounds can be used as therapeutically effective agents against a variety of diseases of the nervous system such as diabetic and toxic neuropathies, peripheral nervous system diseases, Alzheimer disease, Parkinson's disease, stroke, Huntington's disease, motor neuron disease, traumatic nerve injury, multiple sclerosis, dysmyelination and demyelination disorders, and mitochondrial diseases. The Creatine compounds which can be used in the present method include: (1) creatine, creatine phosphate and analogs of these compounds which can act as substrates or substrate analogs for creatine kinase; (2) bisubstrate inhibitors of creatine kinase comprising covalently linked structural analogs of adenosine triphosphate (ATP) and creatine; (3) creatine analogs which can act as reversible or irreversible inhibitors of creatine kinase; and (4) N-phosphorocreatine analogs bearing non-transferable moieties which mimic the N-phosphoryl group.

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BJ	Benin	**IT**	Italy	**PL**	Poland
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CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
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GA	Gabon				

USE OF CREATINE OR CREATINE ANALOGS FOR THE TREATMENT OF DISEASES OF THE NERVOUS SYSTEM

Background of the Invention

Creatine is a compound which is naturally occurring and is found in mammalian brain and other excitable tissues, such as skeletal muscle, retina and heart. It's phosphorylated form, creatine phosphate, also is found in the same organs and is the product of the creatine kinase reaction utilizing creatine as a substrate. Creatine and creatine phosphate can be synthesized relatively easily and are believed to be non-toxic to mammals. Kaddurah-Daouk et al. (WO 92/08456 published May 29, 1992 and WO 90/09192, published August 23, 1990; U.S. 5,321,030; and U.S. 5,324,731) describe methods of inhibiting the growth, transformation and/or metastasis of mammalian cells using related compounds. Examples of compounds described by Kaddurah-Daouk et al. include cyclocreatine, b-guandidino propionic acid, homocyclocreatine, 1-carboxymethyl-2-iminohexahydropyrimidine, guanidino acetate and carbocreatine. These same inventors have also demonstrated the efficacy of such compounds for combating viral infections (U.S. 5,321,030). Elebaly in U.S. Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in muscle tissue. Cohn in PCT publication No. WO94/16687 described a method for inhibiting the growth of several tumors using creatine and related compounds.

The nervous system is an unresting assembly of cells that continually receives information, analyzes and perceives it and makes decisions. The principle cells of the nervous system are neurons and neuroglial cells. Neurons are the basic communicating units of the nervous system and possess dendrites, axons and synapses required for this role. Neuroglial cells consist of astrocytes,

oligodendrocytes, ependymal cells, and microglial cells. Collectively, they are involved in the shelter and maintenance of neurons. The functions of astrocytes are incompletely understood but probably include the provision of biochemical and physical support and aid in insulation of the receptive surfaces of neurons. In addition to their activities in normal brain, they also react to CNS injury by glial scar formation. The principle function of the oligodendrocytes is the production and maintenance of CNS myelin. They contribute segments of myelin sheath to multiple axons.

The ependyma cells react to injury mainly by cell loss. Microglial cells become activated and assume the shape of a macrophage in response to injury or destruction of the brain. These cells can also proliferate and adopt a rod-like form which could surround a tiny focus of necrosis or a dead neuron forming a glial nodule. Microglial degradation of dead neurons is called neuronophagia.

The creatine kinase/creatine phosphate energy system is only one component of an elaborate energy-generating system found in nervous system cells such as, for example, neurons, oligodendrocytes and astrocytes. The components of the creatine energy system include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. The reaction catalyzed by creatine kinase is: $MgADP \pm PCr^{=} + H^{+} \rightleftharpoons MgATP^{=} + Cr$. Some of the functions associated with this system include efficient regeneration of energy in cells with fluctuating and high energy demands, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

The creatine kinase/phosphocreatine system has been shown to be active in neurons, astrocytes, oligodendrocytes and Schwann cells. Manos et al., J. Neurochem. 56:2101-2107 (1991); Molloy et al., J. Neurochem. 59:1925-1932.

The activity of the enzyme has been shown to be up-regulated during regeneration and down-regulated in degenerative states (see, e.g., Annals Neurology 35(3):331-340 (1994); DeLeon et al., J.Neuruosci. Res. 29:437-448 (1991); Orlovskaia et al. Vestnik Rossiiskoi Akademii Meditsinskikh Nauk. 8:34-39 (1992). Burbaeva et al., Shurnal Neuropathologll Psikhiatrii Imeni S-S-Korsakova 90(7):85-87 (1990); Mitochondrial creatine kinase was recently found to be the major constituent of pathological inclusions seen in mitochondrial myopathies. Stadhouders et al., PNAS, 91, pp 5080-5093 (1994).

It is an object of the present invention to provide methods for treatment of diseases that affect cells of the nervous system that utilize the creatine kinase/phosphocreatine system using compounds which modulate the system.

Summary of the Invention

The present invention pertains to methods of treating diseases of the nervous systems in an individual afflicted with such a disease by administering to the afflicted individual an amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate the symptoms of the disease. Compounds which are effective for this purpose include creatine, creatine phosphate, and analogs of creatine or creatine phosphate.

The present invention also provides compositions containing creatine compounds in combination with a pharmaceutically acceptable carrier, and effective amounts of other agents which act on the nervous system, to prophylactically and/or therapeutically treat a subject with a disease of the nervous system. The present invention further pertains to methods of use of creatine compounds in combination with other agents which act on the nervous system for treating diseases of the nervous system.

Packaged drugs for treating subjects having a disease of the nervous system or one who is predisposed to such diseases also are the subject of the present invention. The packaged drugs include a container holding the creatine compound, in combination with a pharmaceutically acceptable carrier, along with instructions for administering the same for the purpose of preventing, ameliorating, arresting or eliminating a disease of the nervous system.

Some of the diseases susceptible to treatment with creatine compounds according to the present invention include, but are not limited to Alzheimer disease, Parkinson's disease, Huntington's disease, motor neuron disease, diabetic and toxic neuropathies, traumatic nerve injury, multiple sclerosis, acute

disseminated encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis, diseases of dysmyelination, mitochondrial diseases, fungal and bacterial infections, migrainous disorders, stroke, aging, dementia, and mental disorders such as depression and schizophrenia.

Brief Description of the Figures

Figure 1 is a graph illustrating the effect of creatine compounds on lesion volumes in mice using the malonate model.

Figure 2 is a graph illustrating the effect of creatine compounds on levels of dopamine, HVA, and DOPAC in mice using the MPTP animal model.

Detailed Description

The methods of the present invention generally comprise administering to an individual afflicted with a disease of the nervous system an amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate symptoms of the disease. Components of the system which can be modulated include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. As used herein, the term "modulate" means to change, affect or interfere with the normal functioning of the component in the creatine kinase/phosphocreatine enzyme system.

Compounds which are particularly effective for this purpose include creatine, creatine phosphate, and analogs thereof which are described in detail below. The term "creatine compounds" will be used herein to include creatine, creatine phosphate, and compounds which are structurally similar to creatine or creatine phosphate, and analogs of creatine and creatine phosphate. The term "creatine compounds" also includes compounds which "mimic" the activity of creatine, creatine phosphate or creatine analogs, i.e., compounds which inhibit or modulate the creatine kinase system. The term "mimics" is intended to include compounds which may not be structurally similar to creatine but mimic the therapeutic activity of creatine, creatine phosphate or structurally similar compounds. The term "inhibitors of creatine kinase system" are compounds which inhibit the activity of the creatine kinase enzyme, molecules that inhibit the creatine transporter or molecules that inhibit the binding of the enzyme to other structural proteins or enzymes or lipids. The term "modulators of the creatine kinase system" are compounds which modulate the activity of the enzyme, or the activity of the transporter of creatine or the ability of other proteins or enzymes or lipids to interact with the system. The term "creatine analog" is intended to include

compounds which are structurally similar to creatine or creatine phosphate, compounds which are art-recognized as being analogs of creatine or creatine phosphate, and/or compounds which share the same or similar function as creatine or creatine phosphate.

The language "treating diseases of the nervous system" is intended to include prevention of the disease, amelioration and/or arrest of a preexisting disease, and the elimination of a preexisting disease. The creatine analogs described herein have both curative and prophylactic effects on disease development and progression.

The language "therapeutically effective amount" is intended to include the amount of the creatine compound sufficient to prevent onset of diseases of the nervous system or significantly reduce progression of such diseases in the subject being treated. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the severity of the symptoms to be treated and the activity of the specific analog selected if an analog is being used. Further, the effective amounts of the creatine compound may vary according to the age, sex and weight of the subject being treated. Thus, a therapeutically effective amount of the creatine compound can be determined by one of ordinary skill in the art employing such factors as described above using no more than routine experimentation in clinical management.

The language "pharmaceutically acceptable carrier" is intended to include substances capable of being coadministered with the creatine compound and which allows the active ingredient to perform its intended function of preventing, ameliorating, arresting, or eliminating a disease(s) of the nervous system. Examples of such carriers include solvents, dispersion media, adjuvants, delay agents and the like. The use of such media and agents for pharmaceutically active

substances is well known in the art. Any conventional media and agent compatible with the creatine compound may be used within this invention.

The term "pharmaceutically acceptable salt" is intended to include art-recognized pharmaceutically acceptable salts. Typically these salts are capable of being hydrolyzed under physiological conditions. Examples of such salts include sodium, potassium and hemisulfate. The term further is intended to include lower hydrocarbon groups capable of being hydrolyzed under physiological conditions, i.e. groups which esterify the carboxyl moiety, e.g. methyl, ethyl and propyl.

The term "subject" is intended to include living organisms susceptible to having diseases of the nervous system, e.g. mammals. Examples of subjects include humans, dogs, cats, horses, cows, goats, rats and mice. The term "subject" further is intended to include transgenic species.

The language "diseases of the nervous system" is intended to include diseases of the nervous system whose onset, amelioration, arrest, or elimination is effectuated by the creatine compounds described herein. Examples of types of diseases of the nervous system include demyelinating, dysmyelinating and degenerative diseases. Examples of locations on or within the subject where the diseases may originate and/or reside include both central and peripheral loci. As the term "disease" is used herein, it is understood to exclude, and only encompass maladies distinct from, neoplastic pathologies and tumors of the nervous system, inschemic injury and viral infections of the nervous system. Examples of types of diseases suitable for treatment with the methods and compounds of the instant invention are discussed in detail below.

Diseases of the Nervous System

Diseases of the nervous system fall into two general categories: (a) pathologic processes such as infections, trauma and neoplasma found in both the nervous system and other organs; and, (b) diseases unique to the nervous system which include diseases of myelin and systemic degeneration of neurons.

Of particular concern to neurologists and other nervous system practitioners are diseases of: (a) demyelination which can develop due to infection, autoimmune antibodies, and macrophage destruction; and, (b) dysmyelination which result from structural defects in myelin.

Diseases of neurons can be the result of: (a) aberrant migration of neurons during embryogenesis and early stage formation; or (b) degenerative diseases resulting from a decrease in neuronal survival, such as occurs in, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease, ischemia-related disease and stroke, and diabetic neuropathy.

Demyelinating Diseases:

Primary demyelination is a loss of myelin sheaths with relative preservation of the demyelinated axons. It results either from damage to the oligodendroglia which make the myelin or from a direct, usually immunologic or toxic attack on the myelin itself. Secondary demyelination, in contrast, occurs following axonal degeneration. The demyelinating diseases are a group of CNS conditions characterized by extensive primary demyelination. They include multiple sclerosis and its variants and perivenous encephalitis. There are several other diseases in which the principal pathologic change is primary demyelination, but which are usually conveniently classified in other categories such as inborn errors of

metabolism, the leukodystrophies, viral disease (progressive multifocal leukoencephalopathy PM), as well as several other rare disorders of unclear etiology.

Multiple Sclerosis (MS)

Multiple sclerosis is a disease of the central nervous system (CNS) that has a peak onset of 30-40 years. It affects all parts of the CNS and causes disability related to visual, sensory, motor, and cerebellar systems. The disease manifestations can be mild and intermittent or progressive and devastating.

The pathogenesis is due to an autoimmune attack on CNS myelin. The treatments available are symptomatic treating spasticity, fatigue, bladder dysfunction, and spasms. Other treatments are directed towards stopping the immunologic attack on myelin. These consist of corticosteroids such as prednisone and methylprednisolone, general immunosuppressants such as cyclophosphamide and azathioprine, and immunomodulating agents such as beta-interferon. No treatments are available to preserve myelin or make it resistant to attacks.

Acute Disseminated Encephalomyelitis

Acute Disseminated Encephalomyelitis usually occurs following a viral infection and is thought to be due to an autoimmune reaction against CNS myelin, resulting in paralysis, lethargy, and coma. It differs from MS by being a monophasic disease whereas MS is characterized by recurrence and chronicity. Treatment consists of administration of steroids.

Acute Necrotizing Hemorrhagic Leukoencephalitis

This is a rare disease that is generally fatal. It is also thought to be mediated by autoimmune attack on CNS myelin that is triggered by a viral infection. Neurologic symptoms develop abruptly with headache, paralysis and coma. Death usually follows within several days. Treatment is supportive.

Leukodystrophies:

These are diseases of the white matter resulting from an error in the myelin metabolism that leads to impaired myelin formation. They are thought of as dysmyelinating diseases, and can become manifest at an early age.

Metachromatic Leukodystrophy: an autosomal recessive (inherited) disorder due to deficiency of the enzyme arylsulfatase A leading to accumulation of lipids. There is demyelination in the CNS and peripheral nervous system leading to progressive weakness and spasticity.

Krabbe's disease: Also inherited as autosomal recessive and due to deficiency of another enzyme: galctocerebroside beta-galactosidase.

Adrenoleukodystrophy and adrenomyeloneuropathy: affect the adrenal glad in addition to the nervous system.

No treatment is available to any of the leukodystrophies except for supportive treatment.

Degenerative Diseases:

There is no good etiology or pathophysiology known for these diseases, and no compelling reason to assume that they all have a similar etiology. Diseases under this category have general similarities. They are diseases of neurons that tend to result in selective impairment, affecting one or more functional systems of neurons while leaving others intact.

Parkinson's Disease:

Parkinson's disease is due to loss of dopaminergic neurones in the substantia nigra of the brain. It is manifested by slowed voluntary movements, rigidity, expressionless face and stooped posture. Several drugs are available to increase dopaminergic function such as levodopa, carbidopa, bromocriptine, pergolide, or decrease cholinergic function such as benztropine, and amantadine. Selegiline is a new treatment designed to protect the remaining dopaminergic neurons.

Spinocerebellar Degenerations

This is a group of degenerative diseases that affects in varying degrees the basal ganglia, brain stem, cerebellum, spinal cord, and peripheral nerves. Patients present symptoms of Parkinsonism, ataxia, spasticity, and motor and sensory deficits reflecting damage to different anatomic areas and/or neuronal systems in the CNS.

Degenerative Disease Affecting Motor Neurons

Included in this category are diseases such as amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy. They are characterized by degeneration of motor neurones in the CNS leading to progressive weakness, muscle atrophy, and

death caused by respiratory failure. Treatments are only symptomatic, there are no available treatments to slow down or stop the disease.

Alzheimer Disease (AD):

This disease is characterized clinically by slow erosion of mental function, culminating in profound dementia. The diagnostic pathologic hallmark of AD is the presence of large numbers of senile plagues and neurofibrillary tangles in the brain especially in neocortex and hippocampus. Loss of specific neuron populations in these brain regions and in several subcortical nuclei correlates with depletion in certain neurotransmitters including acetylcholine. The etiology of AD is still unknown. To date a lot of research has focused on the composition and genesis of the B/A4 amyloid component of senile plagues. Alzheimer's disease is characterized clinically by the slow erosion of intellectual function with the development of profound dementia. There are no treatments that slow the progression.

Huntington Disease (HD):

HD is an autosomal dominant disorder of midlife onset, characterized clinically by movement disorder, personality changes, and dementia often leading to death in 15-20 years. The neuropathologic changes in the brain are centered in the basal ganglia. Loss of a class of projection neurons, called "spiny cells" because of their prominent dendritic spinous processes, is typical. This class of cells contains gamma-aminobutyric acid (GABA), substance P, and opioid peptides. Linkage studies have localized the gene for HD to the most distal band of the short arm of chromosome 4. No treatments are available that have been shown to retard progression of the disease. Experimental studies showing a similarity between neurons that are susceptible to N-methyl d-aspartate (NMDA)

agonists and those that disappear in HD has led to encouraging speculation that NMDA antagonists might prove beneficial. Some recent studies suggest that a defect in brain energy metabolism might occur in HD and enhance neuronal vulnerability to excitotoxic stress.

Mitochondrial Encephalomyopathies:

Mitochondrial encephalomyopathies are a heterogenous group of disorders affecting mitochondrial metabolism. These deficits could involve substrate transport, substrate utilization, defects of the Krebs Cycle, defects of the respiratory chain, and defects of oxidation/phosphorylation coupling. Pure myopathies vary considerably with respect to age at onset, course (rapidly progressive, static, or even reversible), and distribution of weakness (generalized with respiratory failure, proximal more than distal facioscapulohumeral, orbicularis and extraocular muscles with ptosis and progressive external ophthalmoplegia). Patients with mitochondrial myopathies complain of exercise intolerance and premature fatigue.

Peripheral Nervous System Disorders

The peripheral nervous system (PNS) consists of the motor and sensory components of the cranial and spinal nerves, the autonomic nervous system with its sympathetic and parasympathetic divisions, and the peripheral ganglia. It is the conduit for sensory information to the CNS and effector signals to the peripheral organs such as muscle. Contrary to the brain, which has no ability to regenerate, the pathologic reactions of the PNS include both degeneration and regeneration. There are three basic pathological processes: Wallerian degeneration, axonal degeneration and segmental demyelination that could take place.

Some of the neuropathic syndromes include:

Acute ascending motor paralysis with variable sensory disturbance; examples being acute demyelinating neuropathics, infectious mononucleosis with polyneuritis, hepatitis and polyneuritis, toxic polyneuropathies.

Subacute sensorimotor polyneuropathy; examples of acquired axonal neurophathics include paraproteinemias, uremia diabetes, amyloidosis, connective tissue diseases and leprosy. Examples of inherited diseases include mostly chronic demyelination with hypertrophic changes, such as peroneal muscular atrophy, hypertrophic polyneuropathy and Refsum's diseases.

Chronic relapsing polyneuropathy; such as idiopathic polyneuritis porphyria, Beriberi and intoxications.

Mono or multiple neuropathy; such as pressure palsies, traumatic palsies, serum neuritis, zoster and leprosy.

Creatine Compounds Useful For Treating Nervous System Diseases

Creatine compounds useful in the present invention include compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system. Compounds which are effective for this purpose include creatine, creatine phosphate and analogs thereof, compounds which mimic their activity, and salts of these compounds as defined above. Exemplary creatine compounds are described below.

Creatine (also known as N-(aminoiminomethyl)-N-methylglycine; methylglycosamine or N-methyl-guanido acetic acid) is a well-known substance. (See, The Merck Index, Eleventh Edition, No. 2570 (1989).

Creatine is phosphorylated chemically or enzymatically by creatine kinase to generate creatine phosphate, which also is well-known (see, The Merck Index, No. 7315). Both creatine and creatine phosphate (phosphocreatine) can be extracted from animal tissue or synthesized chemically. Both are commercially available.

Cyclocreatine is an essentially planar cyclic analog of creatine. Although cyclocreatine is structurally similar to creatine, the two compounds are distinguishable both kinetically and thermodynamically. Cyclocreatine is phosphorylated efficiently by creatine kinase in the forward reaction both in vitro and in vivo. Rowley, G.L., J. Am. Chem. Soc. 93: 5542-5551 (1971); McLaughlin, A.C. et. al., J. Biol. Chem. 247, 4382-4388 (1972).

The phosphorylated compound phosphocyclocreatine is structurally similar to phosphocreatine; however, the phosphorous-nitrogen (P-N) bond of cyclocreatine phosphate is more stable than that of phosphocreatine. LoPresti, P. and M. Cohn, Biochem. Biophys. Acta 998: 317-320 (1989); Annesley, T. M. and J. B. Walker, J. Biol. Chem. 253; 8120-8125, (1978); Annesley, T.M. and J.B. Walker, Biochem. Biophys. Res. Commun. 74: 185-190 (1977).

Creatine analogs and other agents which act to interfere with the activity of creatine biosynthetic enzymes or with the creatine transporter are useful in the present method of treating nervous system diseases. In the nervous system, there are many possible intracellular, as well as extracellular, sites for the action of compounds that inhibit, increase, or otherwise modify, energy generation through

brain creatine kinase and/or other enzymes which are associated with it. Thus the effects of such compounds can be direct or indirect, operating by mechanisms including, but not limited to, influencing the uptake or biosynthesis of creatine, the function of the creatine phosphate shuttle, inhibiting the enzyme activity, or the activity of associated enzymes, or altering the levels of substrates or products of a reaction to alter the velocity of the reaction.

Substances known or believed to modify energy production through the creatine kinase/phosphocreatine system which can be used in the present method are described below. Exemplary compounds are shown in Tables 1 and 2.

TABLE 1
CREATINE ANALOGS

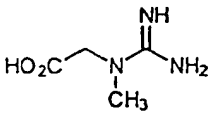
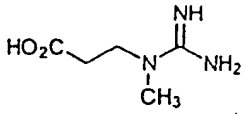
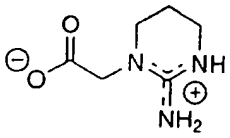
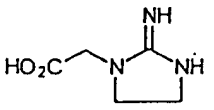
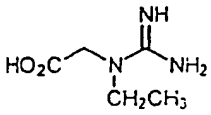
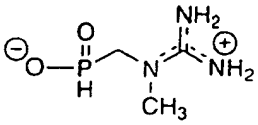
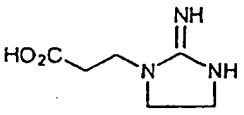
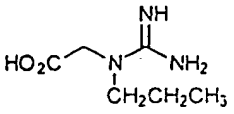
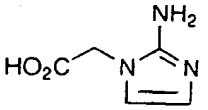
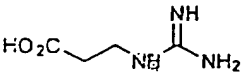
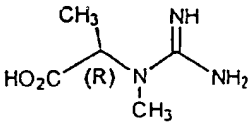
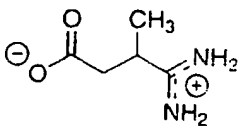
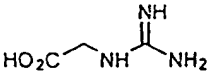
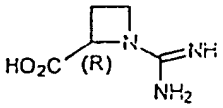
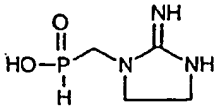
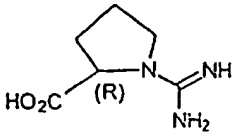
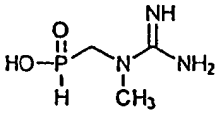
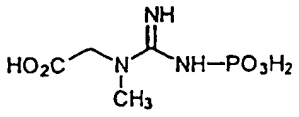
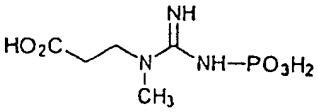
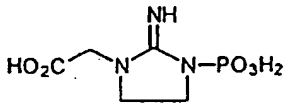


















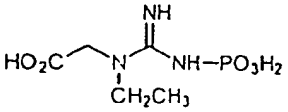
TABLE 2

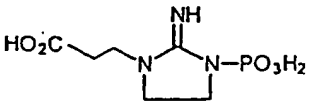
CREATINE PHOSPHATE ANALOGS

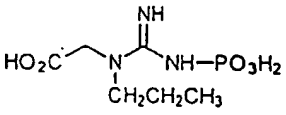


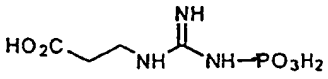


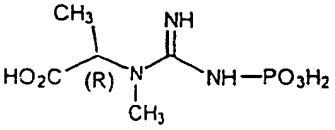


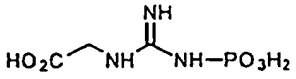


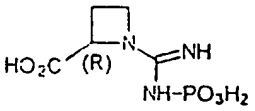


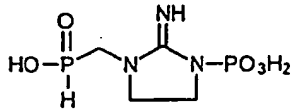


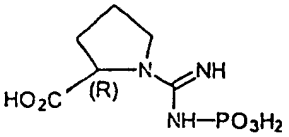


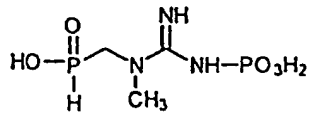


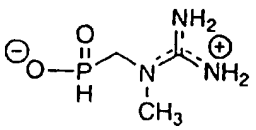


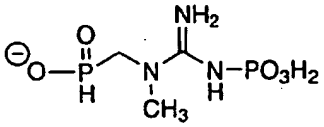












SUBSTITUTE SHEET (RULE 26)

It will be possible to modify the substances described below to produce analogs which have enhanced characteristics, such as greater specificity for the enzyme, enhanced stability, enhanced uptake into cells, or better binding activity.

Compounds which modify the structure or function of the creatine kinase/creatine phosphate system directly or indirectly are useful in preventing and/or treating diseases of the nervous system characterized by up regulation or down regulation of the enzyme system.

In diseases where the creatine kinase/creatine phosphate system is down regulated, for example, uncontrolled firing of neurons, molecules useful for treating these diseases include those that will up regulate the activity, or could support energy (ATP) production for a longer period of time. Examples include creatine phosphate and related molecules that form stable phosphagens which support ATP production over a long period of time.

In diseases where the creatine kinase/creatine phosphate system is up regulated, the molecules that are useful include those that will down regulate the activity and/or inhibit energy production (ATP).

Molecules that regulate the transporter of creatine, or the association of creatine kinase with other protein or lipid molecules in the membrane, the substrates concentration creatine and creatine phosphate also are useful in preventing and/or treating diseases of the nervous system.

Compounds which are useful in the present invention can be inhibitors, substrates or substrate analogs, of creatine kinase, which when present, could modify energy generation or high energy phsphoryl transfer through the creatine kinase/phosphocreatine system. In addition, modulators of the enzymes that work

in conjunction with creatine kinase now can be designed and used, individually, in combination or in addition to other drugs, to make control of the effect on brain creatine kinase tighter.

The pathways of biosynthesis and metabolism of creatine and creatine phosphate can be targeted in selecting and designing compounds which may modify energy production or high energy phosphoryl transfer through the creatine kinase system. Compounds targeted to specific steps may rely on structural analogies with either creatine or its precursors. Novel creatine analogs differing from creatine by substitution, chain extension, and/or cyclization may be designed. The substrates of multisubstrate enzymes may be covalently linked, or analogs which mimic portions of the different substrates may be designed. Non-hydrolyzable phosphorylated analogs can also be designed to mimic creatine phosphate without sustaining ATP production.

A number of creatine and creatine phosphate analogs have been previously described in the literature or can be readily synthesized. Examples are these shown in Table 1 and Table 2. Some of them are slow substrates for creatine kinase.

Tables 1 and 2 illustrate the structures of creatine, cyclocreatine (1-carboxymethyl-2-iminoimidazolidine), N-phosphorocreatine (N-phosphoryl creatine), cyclocreatine phosphate (3-phosphoryl-1-carboxymethyl-2-iminoimidazolidine) and other compounds. In addition, 1-carboxymethyl-2-aminoimidazole, 1-carboxymethyl-2 2-iminomethylimidazolidine, 1-carboxyethyl-2-iminoimidazolidine, N-ethyl-N-amidinoglycine and b-guanidinopropionic acid are believed to be effective.

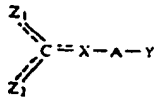
Cyclocreatine (1-carboxymethyl-2-iminoimidazolidine) is an example of a class of substrate analogs of creatine kinase, which can be phosphorylated by creatine kinase and which are believed to be active.

A class of creatine kinase targeted compounds are bi-substrate analogs comprising an adenosine-like moiety linked via a modifiable bridge to a creatine link moiety (i.e., creatine or a creatine analog). Such compounds are expected to bind with greater affinity than the sum of the binding interaction of each individual substrate (e.g., creatine and ATP). The modifiable bridge linking an adenosine-like moiety at the 5'-carbon to a creatine like moiety can be a carbonyl group, alkyl (a branched or straight chain hydrocarbon group having one or more carbon atoms), or substituted alkyl group (an alkyl group bearing one or more functionalities, including but not limited to unsaturation, heteroatom-substituents, carboxylic and inorganic acid derivatives, and electrophilic moieties).

Another class of potential compounds for treating nervous system disorders is designed to inhibit (reversibly or irreversibly) creatine kinase. The analogs of creatine in this class can bind irreversibly to the active site of the enzyme. Two such affinity reagents that have previously been shown to completely and irreversibly inactivate creatine kinase are epoxycreatine Marietta, M.A. and G.L. Kenyon J. Biol Chem. 254: 1879-1886 (1979)) and isoepoxycreatine Nguyen, A.C.K., Ph.D. dissertation in Pharmaceutical Chemistry, (University of California, San Francisco, 1983), pp. 112-205). There are several approaches to enhancing the specificity and hence, the efficacy of active site-targeted irreversible inhibitors of creatine kinase, incorporating an electrophilic moiety. The effective concentration of a compound required for inhibition can be lowered by increasing favorable and decreasing unfavorable binding contacts in the creatine analog.

N-phosphorocreatine analogs also can be designed which bear non-transferable moieties which mimic the N-phosphoryl group. These cannot sustain ATP production.

Some currently preferred creatine compounds of this invention are those encompassed by the general formula I:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$-NHOH, $-NO_2$, $-SO_3H$, $-C(=O)NHSO_2J$ and $-P(=O)(OH)(OJ)$, wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: rromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and

$-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, CHR_1, CR_1, O and S, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_5-C_9 a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) 2 C_5-C_9 a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a C_5-C_9 a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =O, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =O and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) 2 C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: $-CO_2H$-NHOH, $-SO_3H$, $-NO_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1-C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: $-(PO_3)_nNMP$, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic

ring of the base; -[P(=O)(OCH_3)(O)]$_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH_2)]$_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -(PO_3)$_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH_3)(O)]$_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH_2)]$_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -NHR_2, -CH_2R_2 and -NR_2OH, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

Creatine, creatine phosphate and many creatine analogs, and competitive inhibitors are commercially available. Additionally, analogs of creatine may be synthesized using conventional techniques. For example, creatine can be used as the starting material for synthesizing at least some of the analogs encompassed by formula I. Appropriate synthesis reagents, e.g. alkylating, alkenylating or alkynylating agents may be used to attach the respective groups to target sites. Alternatively, reagents capable of inserting spacer groups may be used to alter the creatine structure. Sites other than the target site are protected using conventional protecting groups while the desired sites are being targeted by synthetic reagents.

If the creatine analog contains a ring structure, then the analog may be synthesized in a manner analogous to that described for cyclocreatine (Wang, T., J. Org. Chem. 39:3591-3594 (1974)). The various other substituent groups may be introduced before or after the ring is formed.

Many creatine analogs have been previously synthesized and described (Rowley et al., J. Am. Chem. Soc. 93:5542-5551 (1971); McLaughlin et al., J. Biol. Chem. 247:4382-4388 (1972); Nguyen, A.C.K., "Synthesis and enzyme studies using creatine analogs", Thesis, Dept. of Pharmaceutical Chemistry, Univ. Calif., San Francisco (1983); Lowe et al., J. Biol. Chem. 225:3944-3951 (1980); Roberts et al., J. Biol. Chem. 260:13502-13508 (1985); Roberts et al., Arch.

Biochem. Biophys. 220:563-571 (1983), and Griffiths et al., J. Biol. Chem. 251:2049-2054 (1976)). The contents of all of the forementioned references are expressly incorporated by reference. Further to the forementioned references, Kaddurah-Daouk et al. (WO92/08456; WO90/09192; U.S. 5,324,731; U.S. 5,321,030) also provide citations for the synthesis of a plurality of creatine analogs. The contents of all the aforementioned references and patents are incorporated herein by reference.

Creatine compounds which currently are available or have been synthesized include, for example, creatine, b-guanidinopropionic acid, guanidinoacetic acid, creatine phosphate disodium salt, cyclocreatine, homocyclocreatine, phosphinic creatine, homocreatine, ethylcreatine, cyclocreatine phosphate dilithium salt and guanidinoacetic acid phosphate disodium salt, among others.

Creatine phosphate compounds also can be synthesized chemically or enzymatically. The chemical synthesis is well known. Annesley, T.M. Walker, J.B., Biochem. Biophys. Res. Commun., (1977), 74, 185-190; Cramer, F., Scheiffele, E., Vollmar, A., Chem. Ber., (1962), 95, 1670-1682.

Salts of the products may be exchanged to other salts using standard protocols. The enzymatic synthesis utilizes the creatine kinase enzyme, which is commercially available, to phosphorylate the creatine compounds. ATP is required by creatine kinase for phosphorylation, hence it needs to be continuously replenished to drive the reaction forward. It is necessary to couple the creatine kinase reaction to another reaction that generates ATP to drive it forward. The purity of the resulting compounds can be confirmed using known analytical techniques including ^{1}H NMR, ^{13}CNMR Spectra, Thin layer chromatography, HPLC and elemental analysis.

Utility

In the present invention, the creatine compounds can be administered to an individual (e.g., a mammal), alone or in combination with another compound, for the treatment of diseases of the nervous system. As agents for the treatment of diseases of the nervous system, creatine compounds can interfere with creatine kinase/phosphocreatine functions, thereby preventing, ameliorating, arresting or eliminating direct and/or indirect effects of disease which contribute to symptoms such as paraplegia or memory impairment. Other compounds which can be administered together with the creatine compounds include neurotransmitters, neurotransmitter agonists or antagonists, steroids, corti- costeroids (such as prednisone or methyl prednisone) immunomodulating agents (such as beta-inteferon), immunosuppressive agents (such as cyclophosphamide or azathioprine), nucleotide analogs, endogenous opioids, or other currently clinically used drugs. When co-administered with creatine compounds, these agents can augment interference with creatine kinase/phosphocreatine cellular functions, thereby preventing, reducing, or eliminating direct and/or indirect effects of disease.

A variety of diseases of the nervous system can be treated with creatine or creatine analogs, including but not limited to those diseases of the nervous system described in detail above. Others include bacterial or fungal infections of the nervous system. Creatine or analogs of creatine can be used to reduce the severity of a disease, reduce symptoms of primary disease episodes, or prevent or reduce the severity of recurrent active episodes. Creatine, creatine phosphate or analogs such as cyclocreatine and cyclocreatine phosphate can be used to treat progressive diseases. Many creatine analogs can cross the blood-brain barrier. For example, treatment can result in the reduction of tremors in Parkinson's disease, and other clinical symptoms.

Modes of Administration

The creatine compound can be administered to the afflicted individual alone or in combination with another creatine analog or other agent. The creatine compounds can be administered as pharmaceutically acceptable salts in a pharmaceutically acceptable carrier, for example. The compound may be administered to the subject by a variety of routes, including, but not necessarily limited to, oral (dietary), transdermal, or parenteral (e.g., subcutaneous, intramuscular, intravenous injection, bolus or continuous infusion) routes of administration, for example. An effective amount (i.e., one that is sufficient to produce the desired effect in an individual) of a composition comprising a creatine analog is administered to the individual. The actual amount of drug to be administered will depend on factors such as the size and age of the individual, in addition to the severity of symptoms, other medical conditions and the desired aim of treatment.

Previous studies have described the administration and efficacy of creatine compounds in vivo. For example, creatine phosphate has been administered to patients with cardiac diseases by intravenous injection. Up to 8 grams/day were administered with no adverse side effects. The efficacy of selected creatine kinase substrate analogs to sustain ATP levels or delay rigor during ischemic episodes in muscle has been investigated. On one study, cyclocreatine was fed to mice, rats and chicks, and appeared to be well-tolerated in these animals. Newly hatched chicks were fed a diet containing 1% cyclocreatine. In the presence of antibiotics, the chicks tolerated 1% cyclocreatine without significant mortality, although the chicks grew more slowly than control chicks (Griffiths, G. R. and J. B. Walker, J. Biol. Chem. 251(7): 2049-2054 (1976)). In another study, mice were fed a diet containing 1% cyclocreatine for 10 days (Annesley, T. M. and J. B. Walker, J. Biol. Chem. 253(22): 8120-8125 (1978)). Cyclocreatine has been feed to mice at

up to 1% of their diet for 2 weeks or for over 4 weeks without gross adverse effects. Lillie et al., Cancer Res., 53: 3172-3178 (1993). Feeding animals cyclocreatine (e.g., 1% dietary) has been shown to lead to accumulation of cyclocreatine in different organs in mM concentrations. For example, cyclocreatine was reported to be taken up by muscle, heart and brain in rats receiving dietary 1% cyclocreatine. Griffiths, G. R. and J. B. Walker, J. Biol. Chem. 251(7): 2049-2054 (1976). As shown previously, antiviral activity of cyclocreatine is observed on administering 1% dietary cyclocreatine. Many of the above-referenced studies show that creatine analogs are been shown to be capable of crossing the blood-brain barrier.

The creatine compound can be formulated according to the selected route of administration (e.g., powder, tablet, capsule, transdermal patch, implantable capsule, solution, emulsion). An appropriate composition comprising a creatine analog can be prepared in a physiologically acceptable vehicle or carrier. For example, a composition in tablet form can include one or more additives such as a filler (e.g., lactose), a binder (e.g., gelatin, carboxymethylcellulose, gum arabic), a flavoring agent, a coloring agent, or coating material as desired. For solutions or emulsions in general, carriers may include aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride, solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. In addition, intravenous vehicles can include fluid and nutrient replenishers, and electrolyte replenishers, such as those based on Ringer's dextrose. Preservatives and other additives can also be present. For example, antimicrobial, antioxidant, chelating agents, and inert gases can be added. (See, generally, Remington's Pharmaceutical Sciences, 16th Edition, Mack, Ed., 1980).

The term "administration" is intended to include routes of administration which allow the creatine compounds to perform their intended function(s) of preventing, ameliorating, arresting, and/or eliminating disease(s) of the nervous system in a subject. Examples of routes of administration which may be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, etc.), oral, inhalation, transdermal, and rectal. Depending on the route of administration, the creatine-like compound may be coated with or in a material to protect it from the natural conditions which may detrimentally effect its ability to perform its intended function. The administration of the creatine-like compound is done at dosages and for periods of time effective to reduce, ameliorate or eliminate the symptoms of the nervous system disorder. Dosage regimes may be adjusted for purposes of improving the therapeutic or prophylactic response of the compound. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

In addition, the methods of the instant invention comprise creatine compounds effective in crossing the blood-brain barrier.

The creatine compounds of this invention may be administered alone or as a mixture of creatine compounds, or together with an adjuvant or other drug. For example, the creatine compounds may be coadministered with other different art-recognized moieties such as nucleotides, neurotransmitters, agonists or antagonists, steroids, immunomodulators, immunosuppresants, vitamins, endorphins or other drugs which act upon the nervous system or brain.

Creatine Kinase Isoenzymes in the Brain

Cells require energy to survive and to carry out the multitude of tasks that characterize biological activity. Cellular energy demand and supply are generally balanced and tightly regulated for economy and efficiency of energy use. Creatine kinase plays a key role in the energy metabolism of cells with intermittently high and fluctuating energy requirements such as skeletal and cardiac muscle, brain and neural tissues, including, for example, the retina, spermatozoa and electrocytes. As stated above, the enzyme catalyzes the reversible transfer of the phosphoryl group from creatine phosphate to ADP, to generate ATP. There are multi-isoforms of creatine kinase (CK) which include muscle (CK-MM), brain (CK-BB) and mitochondrial (CK-Mia, CK-Mib) isoforms.

Experimental data suggest that CK is located near the sites in cells where energy generation occurs; e.g., where force generation by motor proteins takes place, next to ion pumps and transporters in membranes and where other ATP-dependent processes take place. It seems to play a complex multi-faceted role in cellular energy homeostasis. The creatine kinase system is involved in energy buffering/energy transport activities. It also is involved in regulating ADP and ATP levels intracellularly as well as ADP/ATP ratios. Proton buffering and production of inorganic phosphate are important parts of the system.

In the brain, this creatine kinase system is quite active. Regional variations in CK activity with comparably high levels in cerebellum were reported in studies using native isoenzyme electrophoresis, or enzymatic CK activity measurements in either tissue extracts or cultured brain cells. Chandler et al. Stroke, 19: 251-255 (1988), Maker et al. Exp. Neurol., 38: 295-300 (1973), Manos et al. J. Neurol. Chem., 56: 2101-2107 (1991). In particular, the molecular layer of the cerebellar cortex contains high levels of CK activity (Maker et al. id. (1973) Kahn

Histochem., 48: 29-32 (1976) consistent with the recent 3'P-NMR findings which indicate that gray matter shows a higher flux through the CK reaction and higher creatine phosphate concentrations as compared to white matter (Cadoux-Hudson et al. FASEBJ., 3: 2660-2666 (1989), but also high levels of CK activity were shown in cultured oligodendrocytes (Manos et al. id. (1991), Molloy et al. J. Neurochem., 59: 1925-1932 (1992), typical glial cells of the white matter. The brain CK isoenzyme CK-BB is the major isoform found in the brain. Lower amounts of muscle creatine kinase (CK-MM) and mitochondrial creatine kinase (CK-Mi) are found.

Localization and Function of CK Isoenzymes
in Different Cells of the Nervous System

Brain CK (CK-BB) is found in all layers of the cerebellar cortex as well as in deeper nuclei of the cerebellum. It is most abundant in Bergmann glial cells (BGC) and astroglial cells, but is also found in basket cells and neurons in the deeper nuclei. Hemmer et al., Eur. J. Neuroscience, 6: 538-549 (1994), Hemmer et al. Dev. Neuroscience, 15: 3-5 (1993). The BGC is a specialized type of astroglial cell. It provides the migratory pathway for granule cell migration from the external to the internal granule cell layer during cerebellar development. Another main function of these cells is the proposed ATP-dependent spatial buffering of potassium ions released during the electrical activity of neurons (Newman et al. Trends Neuroscience, 8: 156-159 (1985), Reichenbach, Acad. Sci New York, (1991), pp. 272-286. Hence, CK-BB seems to be providing energy (ATP) for migration as well as K^+ buffering through regulation of the Na^+/K^+ ATPase. The presence of CK-BB in astrocytes (Manos et al. id. 1991, Hemmer et al. id. 1994, Hemmer et al. id. 1993) may be related to the energy requirements of these cells for metabolic interactions with neurons; e.g., tricarboxylic acid cycle (TCA)

metabolite and neurotransmitter trafficking. Hertz, Can J. Physiol. Pharmacol., 70: 5145-5157 (1991).

The Purkinje neurons of the cerebellum play a very important role in brain function. They receive excitatory input from parallel fibers and climbing fibers, they represent the sole neuronal output structures of the cerebellar cortex. Calcium mediated depolarizations in Purkinje cell dendrites are thought to play a central role in the mechanism of cerebellar motoric learning. Ito Corr. Opin. Neurobiol., 1: 616-620 (1991). High levels of muscle CK (CK-MM) were found in Purkinje neurons. Hemmer et al. id. (1994), Hemmer et al., id. (1993). There is strong evidence to support that CK-MM is directly or indirectly coupled to energetic processes needed for Ca^{++} homeostasis or to cellular processes triggered by this second messenger.

The glomerular structures of the cerebellum contain high levels of CK-BB and mitochondrial CK (CK-Mi). Large amounts of energy are needed in these structures for restoration of potassium ion gradients partially broken down during neuronal excitation as well as for metabolic and neurotransmitter trafficking between glial cells and neurons. Hertz et al., id. (1991). The presence of CK in these structures may be an indication that part of the energy consumed in these giant complexes might be supported by the creatine kinase system.

In neurons, CK-BB is found in association with synaptic vesicles (Friedhoff and Lerner, Life Sci., 20: 867-872 (1977) as well as with plasma membranes (Lim et al., J. Neurochem., 41: 1177-1182 (1983)).

There is evidence to suggest that CK is bound to synaptic vesicles and to the plasma membrane in neurons may be involved in neurotransmitter release as well as in the maintenance of membrane potentials and the restoration of ion gradients

before and after stimulation. This is consistent with the fact that high energy turnover and concomitantly high CK concentrations have been found in those regions of the brain that are rich in synaptic connections; e.g., in the molecular layer of the cerebellum, in the glomerular structures of the granule layer and also in the hippocampus. The observation that a rise in CK levels observed in a fraction of brain containing nerve endings and synapses, parallels the neonatal increase in Na^+/K^+ ATPase is also suggestive that higher levels of creatine phosphates and CK are characteristic of regions in which energy expenditure for processes such as ion pumping are large. Erecinska and Silver, J. Cerebr. Blood Flow and Metabolism, 9: 2-19 (1989). In addition, protein phosphorylation which plays an important role in brain function is also through to consume a sizable fraction of the total energy available in those cells (Erecinska and Silver, id. 1989). Finally, CK, together with nerve-specific enolase belongs to a group of proteins known as slow component b (SCb). These proteins are synthesized in neuronal cell body and are directed by axonal transport to the axonal extremities. Brady and Lasek, Cell, 23: 515-523 (1981), Oblinger et al., J. Neurol., 7: 433-462 (1987) The question of whether CK participates in the actual energetics of axonal transport remains to be answered.

In conclusion, the CK system plays a key role in the energetics of the adult brain. This is supported by ^{31}P NMR magnetization transfer measurements showing that the pseudo first order rate constant of the CK reaction in the direction of ATP synthesis as well as CK flux correlate with brain activity which is measured by EEG as well as by the amount of deoxyglucose phosphate formed in the brain after administration of deoxyglucose. The present inventors have discovered that diseases of the nervous system can be treated by modulating the activity of the creatine kinase/creatine phosphate pathway.

The Role of Creatine Kinase in Treating Diseases of the Nervous System

The mechanisms by which nerve cell metabolites are normally directed to specific cell tasks is poorly understood. It is thought that nerve cells, like other cells, regulate the rate of energy production in response to demand. The creatine kinase system is active in many cells of the nervous system and is thought to play a role in the allocation of high energy phosphate to many diverse neurological processes, such as neurotransmitter biosynthesis, electrolyte flux and synaptic communication. Neurological function requires significant energy and creatine kinase appears to play an important role in controlling the flow of energy inside specialized exitable cells such as neurons. The induction of creatine kinase, the BB isozyme and the brain mitochondrial creatine kinase in particular, results in the generation of a high energy state which could sustain or multiply the pathological process in diseases of the nervous system. Creatine kinase induction also causes release of abnormally elevated cellular energy reserves which appear to be associated with certain diseases of the nervous system. Conversely, suppression of the creatine kinase system, or abberances in it, induce a low energy state which could result in or assist in the death in the process of all the nervous system.

The components of the creatine kinase/phosphocreatine system include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. Some of the functions associated with this system include efficient regeneration of energy in cells with fluctuating and high energy demand, phosphoryl transfer activity, ion transport regulation, cytoskeletal association, nucleotide pool preservation, proton buffering, and involvement in signal transduction pathways. The creatine kinase/phosphocreatine system has been shown to be active in neurons, astrocytes, oligodendrocytes, and Schwann cells.

The activity of the enzyme has been shown to be up-regulated during regeneration and down-regulated in degenerative states, and aberrant in mitochondrial diseases.

Many diseases of the nervous system are thought to be associated with abnormalities in an energy state which could result in imbalanced ion transport neurotransmitter release and result in cell death. It has been reported that defects in mitochondrial respiration enzymes and glycolytic enzymes may cause impairment of cell function.

Without wishing to be bound by theory, it is thought that if the induction or inhibition of creatine kinase is a cause or a consequence of disease, modulating its activity, may block the disease. Modulating its activity would modulate energy flow and affect cell function. Alternatively, another possibility is that creatine kinase activity generates a product which affects neurological function. For example, creatine phosphate may donate a phosphate to a protein to modify its function (e.g., activity, location). If phosphocreatine is such a phosphate donor, creatine analogs which are phosphorylatable or phosphocreatine analogs may competitively inhibit the interaction of phosphocreatine with a target protein thereby directly or indirectly interfering with nervous system functions. Alternatively, phosphorylatable creatine analogs with altered phosphoryl group transfer potential may tie up phosphate stores preventing efficient transfer of phosphate to targets. A neurological disease could be associated with down regulation of creatine kinase activity. In such cases, replenishment of the substrates, e.g., creatine, creatine phosphate or a substrate analog, which could sustain ATP production for an extended of time, with other activators of the enzyme could be beneficial for treatment of the disease.

Ingestion of creatine analogs has been shown to result in replacement of tissue phosphocreatine pools by synthetic phosphagens with different kinetic and

thermodynamic properties. This results in subtle changes of intracellular energy metabolism, including the increase of total reserves of high energy phosphate (see refs. Roberts, J.J. and J.B. Walker, Arch Biochem. Biophys 220(2): 563-571 (1983)). The replacement of phosphocreatine pools with slower acting synthetic phosphagens, such as creatine analogs might benefit neurological disorders by providing a longer lasting source of energy. One such analog, cyclocreatine (1-carboxymethyl-2-aminoimidazolidine) modifies the flow of energy of cells in stress and may interfere with ATP utilization at sites of cellular work.

The pathogenesis of nerve cell death in neurodegenerative diseases is unknown. A significant amount of data has supported the hypothesis that an impairment of energy metabolism may underlie the slow exitotoxic neuronal death. Several studies have demonstrated mitochondrial or oxidative defects in neurodegenerative diseases. Impaired energy metabolism results in decreases in high energy phosphate stores and a deteriorating membrane potential. Under these conditions the voltage sensitive Mg2+block of NMDA receptors is relieved, allowing the receptors to be persistently activated by endogenous concentrations of glutamate. In this way, energy related metabolic defects may lead to neuronal death by a slow exitotoxic mechanism. Recent studies indicate that such a mechanism occurs *in vivo*, and it may play a role in animal models of Huntington's disease and Parkinson's disease.

As discussed in detail above, the creatine kinase/ creatine phosphate energy system is only one component of an elaborate energy- generating system found in the nervous system. The reaction catalyzed by this system results in the rapid regeneration of energy in the form of ATP at sites of cellular work. In the mitochondria the enzyme is linked to the oxidative phosphorylation pathway that has been implicated in diseases of the nervous system. There the enzyme works in the reverse direction where it stores energy in the form of creatine phosphate.

The invention is further illustrated in the following examples, which prove that creatine compounds, represented by creatine itself and the analogue cyclocreatine, are neuroprotective agents in animal models used for neurodegenerative diseases, specifically, Huntington's disease and Parkinson's disease.

Examples

Example 1: Malonate as a model of Huntington's Disease

A series of reversible and irreversible inhibitors of enzymes involved in energy generating pathways have been used to generate animal models for neurodegenerative diseases such as Parkinson's and Huntington's diseases.

Inhibitors of the enzyme succinate dehydrogenase which impact cellular energy state have been used successfully to generate a model for Huntington's disease. Brouillet et. al., J.Neurochem., **60:** 356-359 (1993); Beal et. al., J. Neurosci. **13:** 4181-4192 (1993); Henshaw et. al., Brain Research **647:** 161-166 (1994); Beal et al., J.Neurochem. **61:** 1147-1150 (1993). The enzyme succinate dehydrogenase plays a central role in both the tricarboxilic acid cycle as well as the electron transport chain in the mitochindria. It's reversible inhibitor malonate has recently been evaluated in animals. Intrastriatal injections of malonate in rats was shown to produce dose dependent striatal excitotoxic lesions which are attenuated by both competitive and non competitive NMDA antagonist. Henshaw et. al., Brain Research **647:** 161-166 (1994). Furthermore the glutamate release inhibitor lamotrigine also attenuates the lesions. Co-injection with succinate blocks the lesions, consistent with an effect on succinate dehydrogenase. The lesions are accompanied by a significant reduction in ATP levels as well as significant increase in lactate levels in vivo as shown by chemical shift resonance imaging. Beal et al.,

J.Neurochem. **61:** 1147-1150 (1993). Further more the increases in lactate are greater in older animals consistent with a marked age- of the lesions. Histological studies have shown that the lesion spares NADPH- diaphorase neurons. Somatostatin concentrations were also spared. In vivo magnetic resonance imaging of lesions shows a significant correlation between increasing lesion size and lactate production.

A series of experiments demonstrated that the administration of coenzyme Q_{10} or nicotinamide produced dose dependent protection against the lesions in the malonate animal model. These compounds attenuated ATP depletions produced by malonate in vivo. Further more the co-administration of coenzyme Q_{10} with nicotinamide attenuated the lesions and reduced increases in lactate which occurred after intrastriatal malonate injections.

All of the above mentioned studies supported malonate as a useful model for the neuropathologic and neurochemical features of Huntington's disease. These lesions produced the same pattern of cellular sparing which is seen in Huntington's disease. There is a depletion of striatal spiny neurons yet a relative preservation of the NADPH diaphorase interneurons. Furthermore there is an increase in lactate concentrations which has been observed in Huntington's disease.

The effect of creatine and it's analogue cyclocreatine were evaluated as representatives of creatine compounds in this malonate model for Huntington's disease. Both compounds were administered orally as 1% of the diet. This mode of administration was based on previous studies were significant build up of compounds in organs high in creatine kinase activity such as the muscle and the brain was demonstrated and were 1% cyclocreatine in the diet was shown to

inhibit tumor growth and viral replication. Lillie et al Cancer Research, 53: 3172-3178 (1993); Lillie et. al., Antiviral Research 23: 203-218 (1994).

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing around 300 gms were used in this experiment. Animals were divided into three groups, 7 used as controls, 8 treated with creatine and 8 treated with cyclocreatine. Group one was fed regular chow, whereas the other groups were given chow enriched with 1% creatine or cyclocreatine. The compounds were administered for two weeks prior to the administration of malonate and then for a further week prior to sacrifice. Malonate was dissolved in distilled deionized water and the pH was adjusted to 7.4 with 0.1 M HCl. Intrastriatal injections of 1.5µl of malonate containing 3 µmol were made into the left striatum at the level of the Bregma 2.4 mm lateral to the midline and 4.5 mm ventral to the dura. Animals were sacrificed at 7 days by decapitation and the brains were quickly removed and placed in ice cold 0.9% saline solution. Brains were sectioned at 2mm intervals in a brain mold. Slices were then placed posterior side down in 2% 2,3,5-tiphenyltetrazolium chloride. Slices were stained in the dark at room temperature for 30 minutes and then removed and placed in 4% paraformaldehyde pH 7.3. Lesions, noted by pale staining, were evaluated on the posterior surface of each section using a Bioquant 4 system by an experienced histologist blinded by experimental conditions. These measurements have been validated by comparing them to measurements obtained on adjacent Nissl stain sections to demonstrate the validity of the method. The data are expressed as the means +/- standard error of means (SEM). Statistical comparisons were made by unpaired Student's t test or one- way analysis of variance with the Fisher protected least significant difference (PLSD) test.

As shown in Figure 1, the treatment of animals with creatine produced a significant neuroprotective effect against the intrastriatal injection of malonate.

Cyclocreatine also produced some neuroprotective effect. These results implicate the enzyme creatine kinase in pathways involved in neuronal cell death and supports the therapeutic benefit of the creatine compounds in the treatment of neurodegenerative diseases and mitochondrial encephalopathies. Substantial evidence exists for an impairment of mitochondrial energy metabolism in a number of neurodegenerative diseases. This is particularly true in the case of Huntington's disease. The present lesions model Huntington's disease quite well, thus, the results indicate that creatine compounds are useful in slowing the degenerative process in this illness. Other neurodegenerative diseases which were shown to have underlying defects in energy generation also are expected to be slowed by creatine compounds.

Example 2: MPTP as a model for Parkinson's Disease

MPTP, or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is a neurotoxin which produces a Parkinsonian syndrom in both man and experimental animals. The initial report was by a chemist who was synthesizing and self injecting an opiate analogue. He inadvertently synthesized MPTP and developed profound Parkinsonism. Subsequent pathologic studies showed severe degeneration in the pars compacta of the substantia nigra. A large outbreak subsequently occured in California. These patients developed typical symptoms of Parkinsonism. They also had positron emission tomography done which showed a marked loss of dopaminergic innervation of the stiatum.

Studies of the mechanism of MPTP neurotoxicity show that it involves the generation of a major metabolite, MPP^+. This metabolite is formed by the activity of monoamine oxidase on MPTP. Inhibitors of monoamine oxidase block the neurotoxicity of MPTP in both mice and primates. The specificity of the neurotoxic effects of MPP^+ for dopaminergic neurons appears to be due to the

uptake of MPP^+ by the synaptic dopamine transporter. Blockers of this transporter prevent MPP^+ neurotoxicity. MPP^+ has been shown to be a relatively specific inhibitor of mitochondrial complex I activity. It binds to complex I at the retenone binding site. In vitro studies show that it produces an impairment of oxidative phosphorylation. In vivo studies have shown that MPTP can deplete striatal ATP concentrations in mice. It has been demonstrated that MPP+ administered intrastriatally in rats produces significant depletion of ATP as well as increases in lactate confined to the striatum at the site of the injections. The present inventors have recently demonstrated that coenzyme Q_{10} which enhances ATP production can significantly protect against MPTP toxicity in mice.

The effect of two representative creatine compounds, creatine and cyclocreatine, were evaluated using this model. Creatine and cyclocreatine were administered as 1% formulation in the feed of animals and was administered for three weeks before MPTP treatment. MPTP was administered i.p. at a dose of 15mg/kg every 2hours for five injections. The animals then remained on either creatine or cyclocreaine supplemented diets for 1 week before sacrifice. The mice examined were male Swiss Webster mice weighing 30-35 grams obtained from Taconic Farms. Control groups recieved either normal saline or MPTP hydrochloride alone. MPTP was administered in 0.1 ml of water. The MPTP was obtained from Research Biochemicals. Eight to twelve animals were examined in each group. Following sacrifice the two striatal were rapidly dissected and placed in chilled 0.1 M perchloric acid. Tissue was subsequently sonicated, and aliquots were taken for protein quantification using a fluorometer assay. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were quantified by HPLC with 16 electrode electrochemical detection. Concentrations of dopamine and metabolites were expressed as nmol/mg protein. The statistical significance of differences was determined by one-way ANOVA followed by Fisher PLSDpost-hoc test to compare group means.

The results are shown in Figure 2. Oral administration of either cyclocreatine or creatine significantly protected against DOPAC depletions induced by MPTP. Cyclocreatine was effective against MPTP induced depletions of homovanillic acid. Both administration of creatine and cyclocreatine produce significant neuroprotection against MPTP induced dopamine depletions. The neuroprotective effect produced by cyclocreatine was greater than that seen with creatine alone.

These results indicate that the administration of creatine or cyclocreatine can produce significant neuroprotective effects against MPTP induced dopaminiergic toxicity. These results imply that these compounds are useful for the treatment of Parkinson's disease. The data further establishes the importance of the creatine kinase system in buffering energy and survival of neuronal tissue. Therefor creatine compounds which can sustain energy production in neurons are going to emerge as a new class of protective agents of benefit therapeutically in the treatment of neurodegenerative diseases where impairment of energy has been established.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

CLAIMS

1. A method for treating a subject afflicted with a nervous system disease comprising

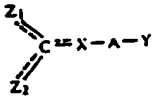
administering to the subject an amount of creatine, creatine phosphate or a creatine analog or a salt thereof compound sufficient to prevent, reduce, ameliorate or eliminate said disease.

2. The method of claim 1 wherein the subject is a mammal.

3. The method of claim 1 wherein the subject is human.

4. A method for treating a subject for diseases of the nervous system comprising:

administering an effective amount of a creatine compound to a subject such that the subject is treated for diseases of the nervous system, wherein the creatine compound is of the general formula:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$, -NHOH, $-NO_2$, $-SO_3H$, $-C(=O)NHSO_2J$ and $-P(=O)(OH)(OJ)$, wherein J is selected from the

group consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5 alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH_2L and -$COCH_2$L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, CHR_1, CR_1, O and S, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having O-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_5-C_9 a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) a C_5-C_9 a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a C_5-C_9 a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =O, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =O and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched

alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having O-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: $-CO_2H$, -NHOH, $-SO_3H$, $-NO_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1-C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: $-(PO_3)_nNMP$, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; $-[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; $-[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected

via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -$(PO_3)_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -NHR_2, -CH_2R_2 and -NR_2OH, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

5. The method of claim 4 wherein the treatment comprises reducing or eliminating symptoms associated with a preexisting disease of the nervous system.

6. The method of claim 4 wherein the treatment comprises preventing the occurrence of diseases of the nervous system within the subject.

7. The method of claim 4 wherein the creatine compound is creatine.

8. The method of claim 4 wherein the creatine compound is creatine phosphate.

9. The method of claim 4 wherein the creatine compound is cyclocreatine.

10. The method of claim 4 wherein the creatine compound is cyclocreatine phosphate.

11. The method of claim 4 wherein the creatine compound is homocyclocreatine.

12. The method of claim 4 wherein the disease of the nervous system is selected from the groups consisting of neuropathies; Alzheimer disease, Parkinson's disease, Huntington's disease, motor neuron disease, traumatic nerve injury, multiple sclerosis, acute disseminated encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis, dysmyelination disease, mitochondrial disease, migrainous disorder, bacterial infection, fungal infection, stroke, aging,

dementia, peripheral nervous system diseases and mental disorders such as depression and schizophrenia.

13. The method of claim 12 wherein the creatine compound is selected from the group consisting of creatine, creatine phosphate, cyclocreatine and cyclocreatine phosphate.

14. The method of claim 4 further comprising coadministering to the subject a neurotransmitter, a neurotransmitter analog, a steroid, an immunomodulating agent, or an immune suppressive agent.

15. The method of claim 4 wherein the subject is treated for diseases of the nervous system by reducing or eliminating symptoms associated with a preexisting diseases of the nervous system.

16. The method of claim 4 wherein the subject is treated for diseases of the nervous system by preventing the occurrence of a disease of the nervous system within the subject.

17. A method for alleviating in a subject being treated for a nervous system disease toxic side effects of drugs used to treat the nervous system diseases, comprising administering to the subject an amount of a creatine, creatine phosphate or a creatine analog, or a salt thereof, sufficient to prevent, reduce, ameliorate or alleviate said toxic side effects.

18. The method of claim 17 wherein the creatine analog is cyclocreatine or cyclocreatine phosphate.

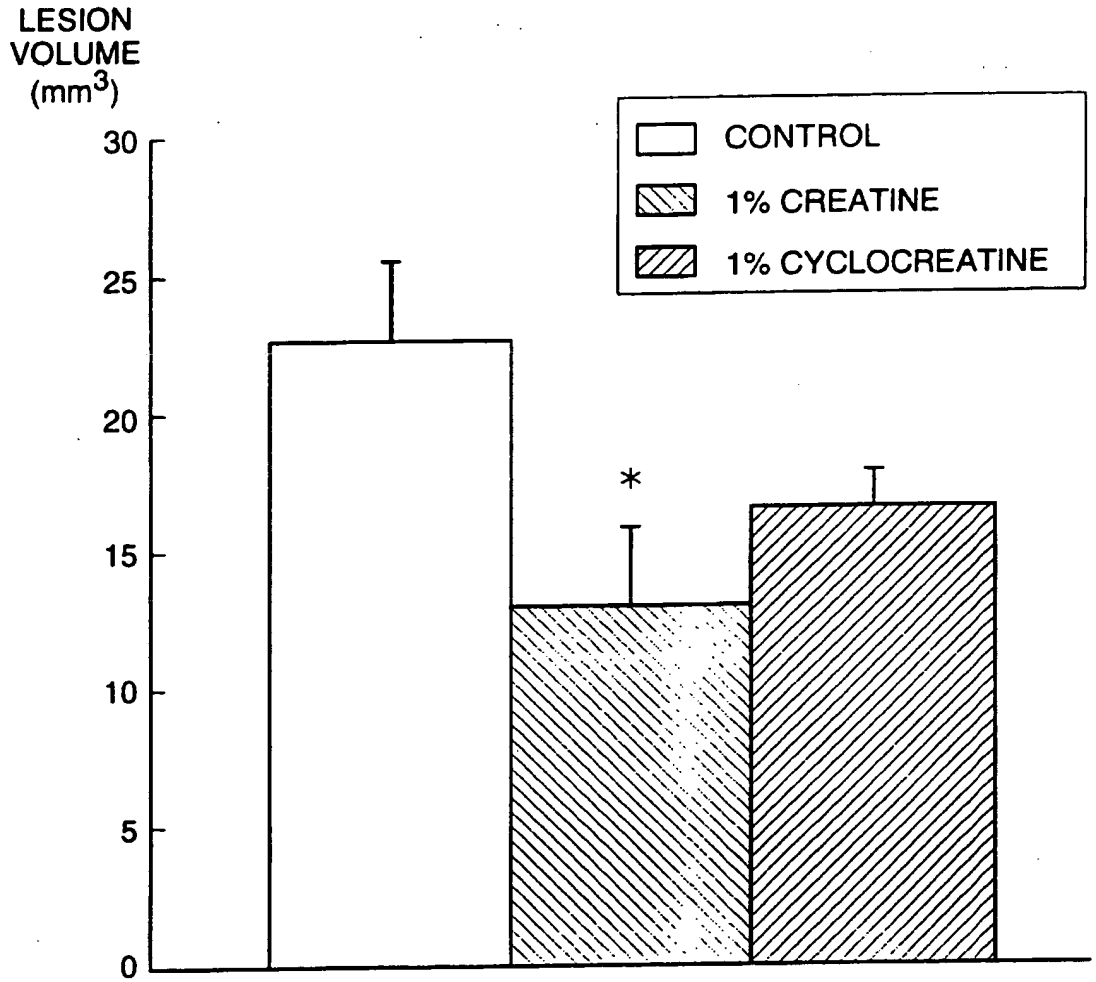

LESION VOLUME (mm^3)
30
25
20
15
10
5
0
CONTROL
1% CREATINE
1% CYCLOCREATINE
*

FIG. 1

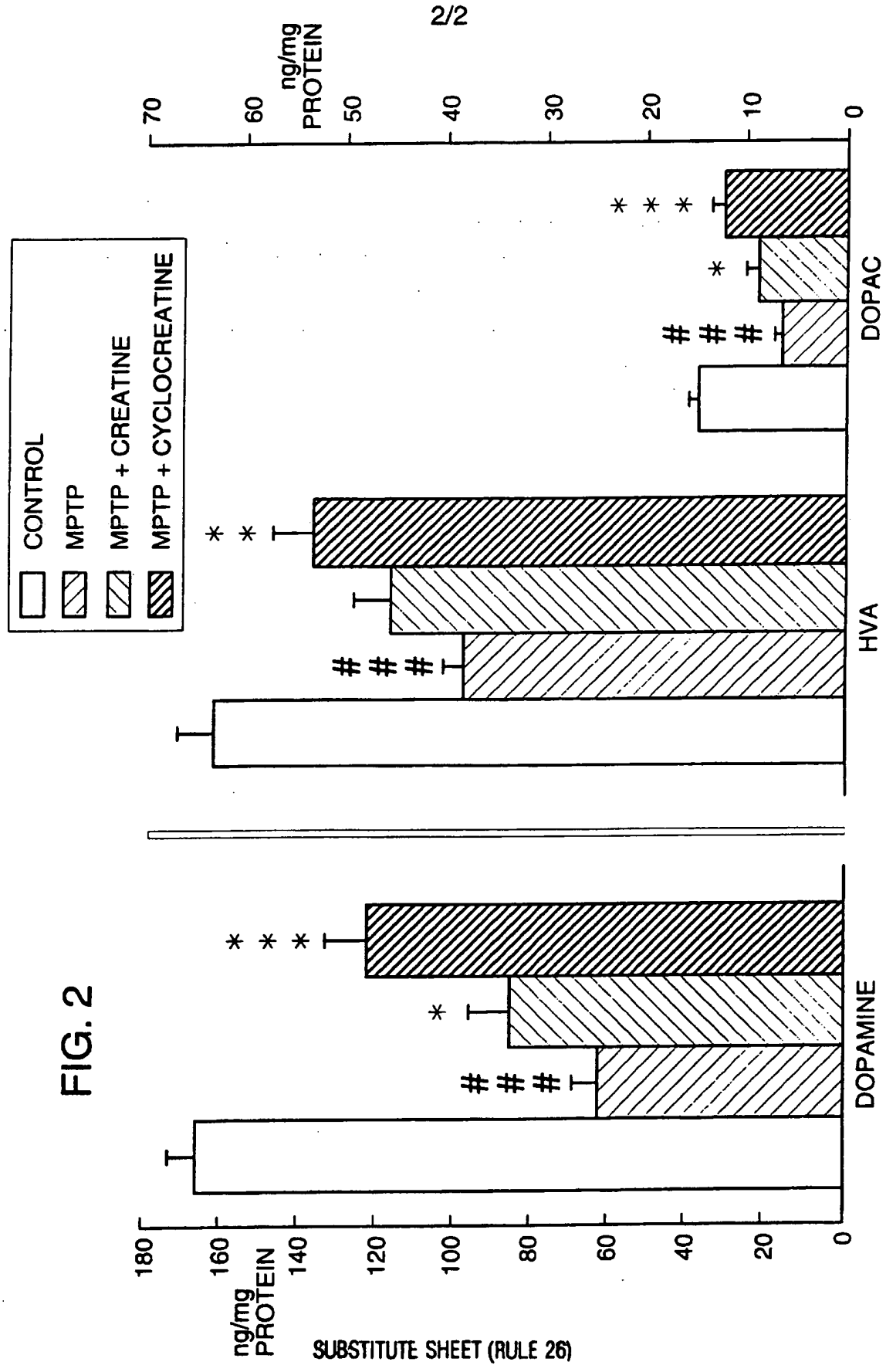

CONTROL
MPTP
MPTP + CREATINE
MPTP + CYCLOCREATINE
ng/mg PROTEIN
0
10
20
30
40
50
60
70
DOPAC
HVA
DOPAMINE
0
20
40
60
80
100
120
140
160
180
ng/mg PROTEIN

FIG. 2

INTERNATIONAL SEARCH REPORT

Int ›onal Application No
PCT/US 95/14567

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/195 A61K31/66 A61K31/675

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MUSCLE & NERVE, vol. 17, no. 10, 1994 pages 1236-1237, XP 000566297 L. HAGENFELDT ET AL. 'Creatine treatment in MELAS.' see the whole document ---	1-5,12, 13
X	WO,A,94 17794 (JENNINGS) 18 August 1994 see page 3; examples ---	1-7,12, 13,16
A	NEUROBIOL. AGING, vol. 15, no. 1, 1994 pages 117-132, XP 000566290 J.W. PETTEGREW ET AL 'Alterations of cerebral metabolism in probable Alzheimer's disease: a preliminary study.' --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 1996	02.05.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Klaver, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 95/14567

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,90 09192 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 23 August 1990 cited in the application	
A	WO,A,92 08456 (AMIRA INC.) 29 May 1992 cited in the application	

1

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US95/14567

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-18 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/14567

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9417794	18-08-94	AU-B-	5890394	29-08-94
		ZA-A-	9400634	14-09-94
WO-A-9009192	23-08-90	AU-B-	1650395	29-06-95
		AU-B-	646799	10-03-94
		AU-B-	5196990	05-09-90
		AU-B-	7287794	24-11-94
		CA-A-	2046607	15-08-90
		EP-A-	0458901	04-12-91
		JP-T-	4506058	22-10-92
		US-A-	5324731	28-06-94
		US-A-	5321030	14-06-94
WO-A-9208456	29-05-92	US-A-	5324731	28-06-94
		AU-B-	8948591	11-06-92
		EP-A-	0557383	01-09-93
		JP-T-	6506666	28-07-94
		US-A-	5321030	14-06-94